

USSN 10/062,970

Page 2

LISTING OF THE CLAIMS

The following is a marked-up version of the Claims pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of the Claims on record. Underlining denotes added text while strikeout denotes deleted text.

1. (Withdrawn) A chemically modified mutant protein, said mutant protein comprising a cysteine residue substituted for a residue other than cysteine in a precursor protein, the substituted cysteine residue being subsequently modified by reacting said cysteine residue with a glycosylated thiosulfonate.
2. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein the protein is an enzyme.
3. (Withdrawn) A chemically modified mutant protein according to claim 2, wherein the enzyme is a protease.
4. (Withdrawn) A chemically modified mutant protein according to claim 3, wherein the protease is a *Bacillus lentus* subtilisin.
5. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein said thiosulfonate comprises an alkylthiosulfonate.
6. (Withdrawn) A chemically modified mutant protein according to claim 5, wherein said alkylthiosulfonate comprises methanethiosulfonate.
7. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein the residue other than cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.
8. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein the residue other than cysteine is in a substrate binding subsite of the protein.

-2-

GC541-3-D1

USSN 10/062,970

Page 3

9. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein the glycosylated thiosulfonate comprises a thiol side chain comprising -S- β -Glc, -S-Et- β -Gal, -S-Et- β -Glc, -S-Et- α -Glc, -S-Et- α -Man, -S-Et-Lac, -S- β -Glc(Ac)₂, -S- β -Glc(Ac)₃, -S- β -Glc(Ac)₄, -S-Et- α -Glc(Ac)₂, -S-Et- α -Glc(Ac)₃, -S-Et- α -Glc(Ac)₄, -S-Et- β -Glc(Ac)₂, -S-Et- β -Glc(Ac)₃, -S-Et- β -Glc(Ac)₄, -S-Et- α -Man(Ac)₃, -S-Et- α -Man(Ac)₄, -S-Et- β -Gal(Ac)₃, -S-Et- β -Gal(Ac)₄, -S-Et-Lac(Ac)₅, -S-Et-Lac(Ac)₆, or -S-Et-Lac(Ac)₇.

10. (Withdrawn) A chemically modified mutant protein according to claim 1, wherein the carbohydrate moiety is a dendrimer moiety.

11. (Withdrawn) A method of producing a chemically modified mutant protein comprising the steps of: (a) providing a precursor protein; (b) substituting an amino acid residue other than cysteine in said precursor protein with a cysteine; (c) reacting said substituted cysteine with a glycosylated thiosulfonate, said glycosylated thiosulfonate comprising a carbohydrate moiety; and (d) obtaining a modified glycosylated protein wherein said substituted cysteine comprises a carbohydrate moiety attached thereto.

12. (Withdrawn) A method according to claim 11, wherein said thiosulfonate comprises an alkylthiosulfonate.

13. (Withdrawn) A method according to claim 12, wherein said alkylthiosulfonate comprises a methanethiosulfonate.

14. (Withdrawn) A method according to claim 11, wherein the protein is an enzyme.

15. (Withdrawn) A method according to claim 14, wherein the enzyme is a protease.

USSN 10/062,970

Page 4

16. (Withdrawn) A method according to claim 15, wherein the protease is a *Bacillus lentus subtilisin*.

17. (Withdrawn) A method according to claim 11, wherein the amino acid residue other than cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.

18. (Withdrawn) A method according to claim 11, wherein the amino acid residue other than cysteine is in a substrate binding subsite of the protein.

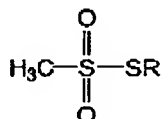
19. (Withdrawn) A method according to claim 11, wherein the glycosylated thiosulfonate comprises a thiol side chain comprising -S-β-Glc, -S-Et-β-Gal, -S-Et-β-Glc, -S-Et-α-Glc, -S-Et-α-Man, -S-Et-Lac, -S-β-Glc(Ac)₂, -S-β-Glc(Ac)₃, -S-β-Glc(Ac)₄, -S-Et-α-Glc(Ac)₂, -S-Et-α-Glc(Ac)₃, -S-Et-α-Glc(Ac)₄, -S-Et-β-Glc(Ac)₂, -S-Et-β-Glc(Ac)₃, -S-Et-β-Glc(Ac)₄, -S-Et-α-Man(Ac)₃, -S-Et-α-Man(Ac)₄, -S-Et-β-Gal(Ac)₃, -S-Et-β-Gal(Ac)₄, -S-Et-Lac(Ac)₅, -S-Et-Lac(Ac)₆, or -S-Et-Lac(Ac)₇.

20. (Withdrawn) A method according to claim 11, wherein the carbohydrate moiety is a dendrimer moiety.

USSN 10/062,970

Page 5

21. (Currently Amended) A glycosylated thiosulfonate comprising:



wherein R comprises β -Glc, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Gal}$, $\text{Et } \beta\text{-Glc}$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Glc}$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Man}$, $\text{Et } \underline{\text{O}}\text{-Lac}$, $\beta\text{-Glc}(\text{Ac})_2$, $\beta\text{-Glc}(\text{Ac})_3$, $\beta\text{-Glc}(\text{Ac})_4$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Glc}(\text{Ac})_2$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Glc}(\text{Ac})_3$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Glc}(\text{Ac})_4$, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Glc}(\text{Ac})_2$, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Glc}(\text{Ac})_3$, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Glc}(\text{Ac})_4$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Man}(\text{Ac})_3$, $\text{Et } \underline{\text{O}}\text{-}\alpha\text{-Man}(\text{Ac})_4$, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Gal}(\text{Ac})_3$, $\text{Et } \underline{\text{O}}\text{-}\beta\text{-Gal}(\text{Ac})_4$, $\text{Et } \underline{\text{O}}\text{-Lac}(\text{Ac})_5$, $\text{Et } \underline{\text{O}}\text{-Lac}(\text{Ac})_6$, or $\text{Et } \underline{\text{O}}\text{-Lac}(\text{Ac})_7$.

22. (Withdrawn) A method of modifying the functional characteristics of a protein comprising:

providing a protein and

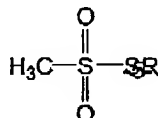
reacting the protein with a glycosylated thiosulfonate reagent under conditions effective to produce a glycoprotein with altered functional characteristics as compared to the protein.

23. (Withdrawn) A method according to claim 22, wherein the protein is an enzyme.

24. (Withdrawn) A method according to claim 23, wherein the enzyme is a protease.

25. (Withdrawn) A method according to claim 24, wherein the protease is a *Bacillus lentus subtilisin*.

26. (Withdrawn) A method according to claim 22, wherein the glycosylated thiosulfonate comprises:



GC541-3-D1

USSN 10/062,970

Page 6

wherein R comprises - β -Glc, -Et- β -Gal, -Et- β -Glc, -Et- α -Glc, -Et- α -Man, -Et-Lac, - β -Glc(Ac)₂, - β -Glc(Ac)₃, - β -Glc(Ac)₄, -Et- α -Glc(Ac)₂, -Et- α -Glc(Ac)₃, -Et- α -Glc(Ac)₄, -Et- β -Glc(Ac)₂, -Et- β -Glc(Ac)₃, -Et- β -Glc(Ac)₄, -Et- α -Man(Ac)₃, -Et- α -Man(Ac)₄, -Et- β -Gal(Ac)₃, -Et- β -Gal(Ac)₄, -Et-Lac(Ac)₅, -Et-Lac(Ac)₆, or -Et-Lac(Ac)₇.

27. (Withdrawn) A method of determining the structure-function relationships of chemically modified mutant proteins comprising:

providing first and second chemically modified mutant proteins according to claim 1, wherein the glycosylation pattern of the second chemically modified mutant protein is different from the glycosylation pattern of the first chemically modified mutant protein;

evaluating a functional characteristic of the first and second chemically modified mutant proteins; and

correlating the functional characteristic of the first and second chemically modified mutant proteins with the structures of the first and second chemically modified mutant proteins.

28. (Withdrawn) A method according to claim 27, wherein the protein is an enzyme.

29. (Withdrawn) A method according to claim 28, wherein the enzyme is a protease.

30. (Withdrawn) A method according to claim 29, wherein the protease is a *Bacillus lentus* subtilisin.

31. (Withdrawn) A method according to claim 27, wherein the residue other than cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.

USSN 10/062,970

Page 7

32. (Withdrawn) A method according to claim 27, wherein the residue other than cysteine is in a substrate binding subsite of the protein.

33. (Withdrawn) A method according to claim 27, wherein the glycosylated thiosulfonate comprises a thiol side chain comprising -S- β -Glc, -S-Et- β -Gal, -S-Et- β -Glc, -S-Et- α -Glc, -S-Et- α -Man, -S-Et-Lac, -S- β -Glc(Ac)₂, -S- β -Glc(Ac)₃, -S- β -Glc(Ac)₄, -S-Et- α -Glc(Ac)₂, -S-Et- α -Glc(Ac)₃, -S-Et- α -Glc(Ac)₄, -S-Et- β -Glc(Ac)₂, -S-Et- β -Glc(Ac)₃, -S-Et- β -Glc(Ac)₄, -S-Et- α -Man(Ac)₃, -S-Et- α -Man(Ac)₄, -S-Et- β -Gal(Ac)₃, -S-Et- β -Gal(Ac)₄, -S-Et-Lac(Ac)₅, -S-Et-Lac(Ac)₆, or -S-Et-Lac(Ac)₇.

34. (Withdrawn) A method according to claim 27, wherein the carbohydrate moiety is a dendrimer moiety.

35. (Withdrawn) A method of determining the structure-function relationships of chemically modified mutant proteins comprising:

providing first and second chemically modified mutant proteins according to claim 1, wherein at least one different cysteine residue in the second chemically modified mutant enzyme is modified by reacting said cysteine residue with a glycosylated thiosulfonate;

evaluating a functional characteristic of the first and second chemically modified mutant proteins; and

correlating the functional characteristic of the first and second chemically modified mutant proteins with the structures of the first and second chemically modified mutant proteins.

36. (Withdrawn) A method according to claim 35, wherein the protein is an enzyme.

37. (Withdrawn) A method according to claim 36, wherein the enzyme is a protease.

USSN 10/062,970

Page 8

38. (Withdrawn) A method according to claim 37, wherein the protease is a *Bacillus lentus subtilisin*.

39. (Withdrawn) A method according to claim 35, wherein the residue other than cysteine is an amino acid selected from the group consisting of asparagine, leucine, and serine.

40. (Withdrawn) A method according to claim 35, wherein the residue other than cysteine is in a substrate binding subsite of the protein.

41. (Withdrawn) A method according to claim 35, wherein the glycosylated thiosulfonate comprises a thiol side chain comprising -S- β -Glc, -S-Et- β -Gal, -S-Et- β -Glc, -S-Et- α -Glc, -S-Et- α -Man, -S-Et-Lac, -S- β -Glc(Ac)₂, -S- β -Glc(Ac)₃, -S- β -Glc(Ac)₄, -S-Et- α -Glc(Ac)₂, -S-Et- α -Glc(Ac)₃, -S-Et- α -Glc(Ac)₄, -S-Et- β -Glc(Ac)₂, -S-Et- β -Glc(Ac)₃, -S-Et- β -Glc(Ac)₄, -S-Et- α -Man(Ac)₃, -S-Et- α -Man(Ac)₄, -S-Et- β -Gal(Ac)₃, -S-Et- β -Gal(Ac)₄, -S-Et-Lac(Ac)₅, -S-Et-Lac(Ac)₆, or -S-Et-Lac(Ac)₇.

42. (Withdrawn) A method according to claim 35, wherein the carbohydrate moiety is a dendrimer moiety.